AS Unit 1: Basic Biochemistry and Cell Organisation

Name:	Date:

Topic 1.5 Medical and Industrial Applications of Enzymes – Page 1

l. Introduction to Enzymes

		Completed
1.	Read the notes on the medical and industrial applications of enzymes.	
2.	Read the following: • Rowlands p51-54 • Toole p604-606	
3.	Read through the BioFactsheet and look carefully at the questions.	

Medical and Industrial Applications of Enzymes

Enzymes were first used on a large scale by the textile industry. Thread used for weaving used to be protected by coating it with starch paste – useful as it made the thread slippery and therefore weaving easier. After weaving the starch had to be removed and this used to be done with various chemicals such as acids but they often discoloured the cloth. In the early twentieth century the industry starting using enzyme extracts from an animal's pancreas. At around the same time the leather industry started to use proteases to remove animal hair from the skins. By 1945 large scale production of enzymes was commonplace.

Industrial Uses of Enzymes Today
The ability of enzymes to catalyse specific chemical reactions at body temperature makes them commercially useful tools. Some examples of enzyme use are given below:

Enzyme	Reaction	Source of Enzyme	Application
Glucose isomerase	Converts glucose to sucrose	Fungi	Production of high fructose syrups used in the food industry
Proteases	Digest protein	Bacteria	Washing powder
Urokinase	Breaks down blood clots	Human urine	Removes blood clots in heart disease patients
Glucose oxidase	Oxidises glucose	Fungi	Used to test for blood glucose in for people with diabetes
Lysozyme	Breaks 1-4 glycosidic bonds	Hen egg white	Disrupts bacterial cell walls
Endonucleases	Breaks DNA into fragments	Bacteria	Used in genetic manipulation techniques eg gene transfer, DNA fingerprinting

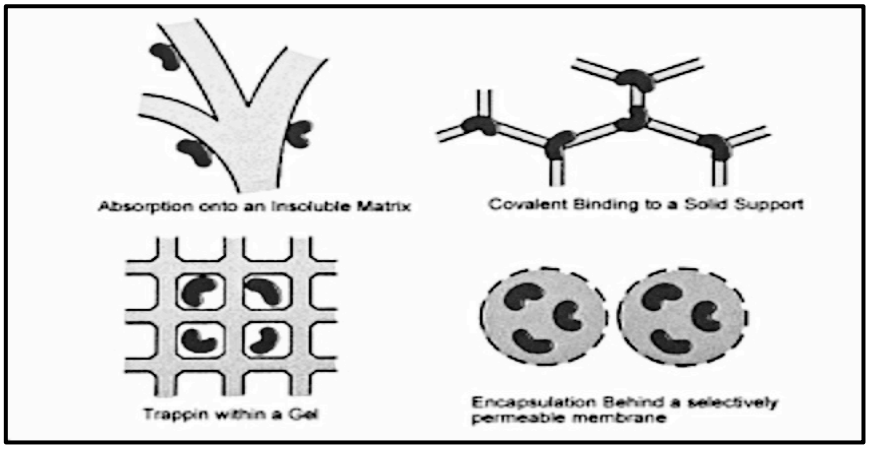
Enzymes used in industry can either be intracellular or extracellular in origin. Extracellular enzymes are easier to isolate, as the organisms producing it can be grown and they will secrete it into a growth medium. Intracellular enzymes are more tricky to obtain as the organisms have to be grown and then the cells need to be broken open to extract the enzymes.

Immobilised Enzymes

In an industrial process controlled by an enzyme, the enzyme is usually the most expensive component. Enzymes like most catalysts are not used up in the reaction and therefore they can theoretically be reused.

One disadvantage of simply adding an enzyme to the substrate in solution is that it might then be difficult to separate the enzyme from the product/s. This enzyme many not be easily re-used which would be expensive.

An alternative is to use immobilised enzymes. Immobilised enzymes are bound to a surface so that they are not allowed to dissolve and usually they cannot move. There are several ways of holding and immobilising the enzymes. They are usually bound to an inert support or matrix.



Enzyme immobilization also has the additional benefit of often making the enzyme able to tolerate a wider range of conditions i.e. tolerate a wider pH range or still be stable in structure at temperatures above their optimum. This later fact means that often more than one enzyme can be immobilised onto the same matrix. Enzyme immobilization is important in **biosensors**.

Biosensors
Biosensors are instruments that can detect a **specific** molecule or metabolite in a mixture of molecules or body fluids. They can detect molecules at even very low concentrations and give quantitative readings whilst at the same time not contaminating the product because the enzymes are immobilised.

Their specificity is due to the presence of an enzyme. To use enzymes as biosensors they need to be:

- Capable of immobilisation
- Able to withstand changes in pH or temperature
- Specific to the molecule being detected

A simple biosensor involves the immobilization of the enzyme glucose oxidase which is specific for the substrate glucose.

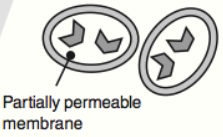
Putting Enzymes to Use

Depending on the way in which the desired end-product is produced, enzymes may be used as crude whole cell preparations or as cell-free enzyme extracts. Whole cell preparations are cost effective, and appropriate when the processes involved in production of the end product are complex, as in waste treatment and the production of semi-synthetic antibiotics. Cell free enzyme extracts are more expensive to produce, but can be a more efficient option overall. To reduce costs and improve the efficiency of product production, enzymes are sometimes immobilised within a matrix of some kind and the reactants are passed over them. The various methods by which enzymes are put to work are compared in the diagram below.

Industrial enzymes

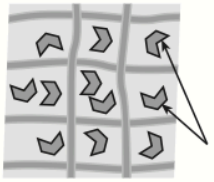
	Advantages	Disadvantages
Cell free enzyme extract Enzyme is used in solution	There is generally a high level of enzyme activity when the enzymes are free in solution.	The enzyme may be washed away after use. The end-product is not enzyme free and may require purification.
Immobilised enzyme Enzyme is held in an inert material	The enzymes can be used repeatedly and recovered easily (this reduces costs). The enzyme-free end-product is easily harvested. The enzymes are more stable due to the protection of a matrix. The life of some enzymes, e.g. proteases, is extended by immobilisation.	The entrapment process may reduce the enzyme activity (more enzyme will be needed). Some methods offering high stability (e.g. covalent bonding) are harder to achieve. Immobilisation can be costly.
Whole cell preparation Whole cells may be immobilised	Useful for enzymes that are unstable or inactivated when outside the cell. Useful for complex processes utilising more than one intracellular enzyme.	Less expensive and more rapid than first producing a pure enzyme extract. Some of the substrate is used for microbial growth, so the process is less efficient overall.

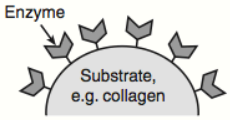
Methods of Enzyme Immobilisation



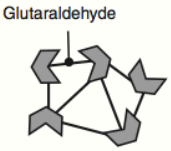
Micro-encapsulation
The enzyme is held within a membrane, or within alginate or polyacrylamide capsules.

Lattice entrapment
Enzyme is trapped in a gel lattice, e.g. silica gel. The substrate and reaction products diffuse in and out of the matrix.


Enzymes trapped in a gel lattice



Covalent attachment
Enzyme is covalently bonded to a solid surface e.g. collagen or a synthetic polymer.



Direct cross-linking
Glutaraldehyde is used to cross-link the enzymes. They then precipitate out and are immobilised without support.

1. (a) Explain one benefit of using a cell free enzyme extract to produce a high-value end-product:

   _____

   (b) Identify one factor that might be important when deciding not to use a cell free extract:

   _____

2. (a) Describe two benefits of using immobilised enzymes (rather than enzymes in solution) for industrial processes:

   _____

   _____

   (b) Describe a disadvantage associated with the use of immobilised enzymes: _____

   _____

   (c) Describe a factor that would affect the rate of end-product harvest from immobilised enzymes:

   _____

3. The useful life of protease enzymes is extended when they are immobilised (as opposed to being in solution). Using what you know of enzyme structure, explain why immobilisation has this effect in this case:

   _____

   _____

4. Suggest why immobilisation would reduce the activity of certain enzymes: _____

   _____

   _____

Commercial applications of enzymes

Analysis

Glucose oxidase
The reaction catalysed by glucose oxidase is

$$\beta\text{-D-glucose} + O_2 \longrightarrow \text{gluconic acid} + H_2O_2$$

The quick and accurate measurement of glucose is of great importance both medically (in sufferers from diabetes, for example) and industrially (in fermentation reactions, for example). A simple quantitative procedure can be devised by coupling the production of hydrogen peroxide to the activity of the enzyme **peroxidase.**

$$DH_2 + H_2O_2 \xrightarrow{\text{peroxidase}} 2H_2O + D$$

chromagen a hydrogen donor (colourless) → coloured compound (colour)

Peroxidase can oxidise an organic chromagen (DH_2) to a coloured compound (D) utilising the hydrogen peroxide – the amount of the coloured compound D produced is a direct measure of the amount of glucose which has reacted. It can be measured quantitatively using a colorimeter or, more subjectively, by comparison with a colour reference card.

Clinistix

This method of glucose analysis is **highly specific** and has the enormous advantage over chemical methods in that this specificity allows glucose to be assayed **in the presence of other sugars**, e.g. in a biological fluid such as blood or urine, without the need for an initial separation.

Both of the enzymes glucose oxidase and peroxidase, and the chromagen DH_2, can be immobilised on a cellulose fibre pad. This forms the basis of the glucose dipsticks ('Clinistix') which were developed to enable diabetics to monitor their own blood or urine glucose levels.

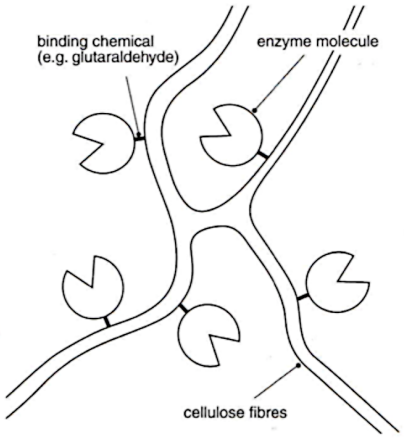
Textiles **Subtilisin** is a bacterial protease (protein → amino acids) which is used in bioactive detergents to remove protein stains from clothes.

Food production **Amylase** and **glucose isomerase** convert starch → high fructose syrups. these syrups have enhanced sweetening power and lowered energy content.

There are many applications of enzyme technology to industry. Enzyme technology has several advantages over 'whole-organism' technology.

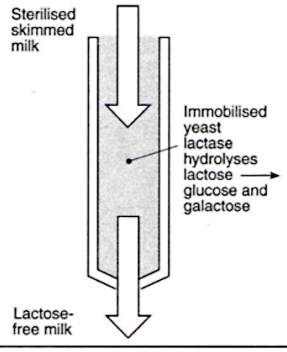
1. **No loss of substrate due to increased biomass.** For example, when whole yeast is used to ferment sugar to alcohol it always 'wastes' some of the sugar by converting it into cell wall material and protoplasm for its own growth.
2. **Elimination of wasteful side reactions.** Whole organisms may convert some of the substrate into irrelevant compounds or even contain enzymes for degrading the desired product into something else.
3. **Optimum conditions for a particular enzyme may be used.** These conditions may not be optimal for the whole organism – in some organisms particular enzymes might be working at less than maximum efficiency.
4. **Purification of the product is easier.** This is especially true using immobilised enzymes.

Medicine



Enzyme immobilisation
Immobilisation means physically or chemically trapping enzymes or cells onto surfaces or inside fibres. The benefits can be considerable:

- the same enzyme molecules can be used again and again, since they are not lost;
- the enzyme does not contaminate the end product;
- the enzymes may be considerably more stable in immobilised form – for example, glucose isomerase is stable at 65 °C when immobilised.



An important medical application of an immobilised enzyme

Some adults are **lactose-intolerant** since they lack an intestinal lactase, and undigested lactose in the gut is metabolised by bacteria causing severe abdominal pain and diarrhoea.

Milk is an important dietary component and can be made **lactose-free** by passage down a column packed with **yeast lactase** immobilised on fibres of cellulose acetate.

Bio Factsheet

Number 47

The Economic Importance of Enzymes

Enzymes are important in commercial processes because they accelerate specific chemical reactions to produce a useful product or effect. Enzymes are sometimes used when still retained within cells; other processes require the enzyme to be extracted from the parent cells and purified. In both cases the efficiency of the process may be increased by trapping the cells or purified enzyme within an insoluble agent. This is called enzyme immobilisation. This Factsheet outlines the processes of enzyme extraction, purification and immobilisation, and summarises the functions of commercial enzymes.

Naming of enzymes

It is important to understand how enzymes are named if their commercial use is to be understood. The names of many enzymes end in the suffix – ase. The first part of the word often indicates the type of molecule on which the enzyme acts (the substrate – see Table 1).

Table 1.

Enzyme	Substrate
proteases	proteins
amylases	carbohydrates
lipases	lipids
cellulases	cellulose
lactases	lactose
pectinases	pectin

Exam Hint: *Make sure you know what categories of enzymes are in your syllabus.*

Enzyme technology

The process of incorporating the enzyme into the commercial process is enzyme technology. The function of this technology is to make the manufacturing process more productive by, for example, reducing waste, removing contaminants and maximising the quantity of product produced in terms of the cost of raw materials.

Enzyme production

Sources of commercial enzymes

A wide variety of microorganisms – bacteria, fungi and yeast – are used as source material. The bacteria Bacillus and fungi Aspergillus are particularly significant. Microorganisms can be genetically engineered to produce higher yields or different types of enzymes.

Purification

The parent cells are first disrupted by physical or chemical means, e.g. grinding or by the addition of alkalis. The cell debris is then removed by filtration or centrifugation. Different enzymes are then precipitated from solution using ammonium sulphate, which is inexpensive and of low toxicity. Further purification can involve a variety of separation techniques such as chromatography, filtration and electrophoresis.

Chromatography and electrophoresis

Chromatography separates the different components of a mixture using a solvent moving through a stationary medium such as chromatography paper. The various components move along the medium at different rates and can then be individually isolated. Electrophoresis achieves a similar effect when an electric current is passed through an appropriate medium and produces positive and negative ends. The different components move through the medium at different rates depending on their size and charge.

Immobilisation

In immobilisation the enzyme is held in a column of insoluble agent through which the substrate flows. Whole cells or extracted enzymes can be immobilised. The binding agent can be organic (e.g. agar gel, cellulose) or inorganic (e.g. porous alumina, porous glass). The mechanism of binding can be physical, as in entrapment within a lattice, or chemical, in which the enzyme forms chemical bonds with the immobilising agent.

An alternative to immobilisation is to operate a so-called batch system in which enzyme and substrate react together in a closed vessel from which the product is periodically extracted.

Examples of commercial enzyme functions

Detection and measurement of glucose

Enzymes can be used to detect molecular markers of clinical importance. One widely quoted example is the detection and measurement of glucose. The amount of glucose in blood or urine is a crucial indicator in the diagnosis and treatment of diabetes mellitus.

Diabetes mellitus

This is caused by a deficiency of the hormone insulin, which is secreted by the pancreas. Well-known symptoms are a high concentration of glucose in the blood and the presence of glucose in the urine.

Glucose can be detected using the enzyme glucose oxidase in a biosensor. A biosensor is an instrument used, as the name indicates, to detect – hence sensor – molecules outside the instrument. The instrument uses some kind of biological system (bio-), for example, an enzyme, to detect this molecule. The reaction produced in the biological system is then converted into electrical activity by a transducer.

Transducer

This word is now frequently used in biology textbooks. The standard dictionary definition can be quite wide, but in biology it is often used to indicate the conversion of some kind of stimulus energy into electrical energy. A rough analogy of biosensors in the human body might be the sensory receptors in the nose, which generate electrical signals in nerve cells as a result of the detection of air-borne chemicals.

A glucose biosensor uses glucose oxidase as its biological system. This enzyme catalyses the reaction between glucose and oxygen to form gluconic acid and hydrogen peroxide. The glucose can then be detected and measured by the quantity of oxygen consumed or gluconic acid produced. These cause changes in the electrical signal generated by the transducer.

An alternative use of glucose oxidase is to use it on a cellulose fibre pad, e.g. 'Clinistix'. The hydrogen peroxide generated as a result of the activity of the glucose oxidase reacts with a colourless compound to form a coloured compound, which indicates the presence of glucose. A second enzyme, peroxidase, also impregnated on the pad, is necessary for the colour reaction.

Genetic Engineering
Genetic engineering depends on three major groups of enzymes which help to copy, cut and join DNA molecules. The enzymes are called reverse transcriptase, restriction endonucleases and DNA ligases respectively.

DNA molecules
DNA means deoxyribonucleic acid. Each chromatid of a chromosome is a single DNA molecule.

Reverse transcriptase is an enzyme which helps construct complementary sections of DNA from messenger RNA. This is an invaluable method of building that section of the DNA which contains the gene required for cloning.

Restriction endonucleases cut DNA molecules at specific base sequences leaving 'sticky ends' - the base sequence which is exposed is then joined to the corresponding base sequence on the other piece of DNA.

DNA ligases glue the DNA fragments together at the sticky ends to make **recombinent DNA** sections into plasmids, which are circular loops of DNA extracted from bacterial cells. The modified plasmids are then inserted into other microorganisms which are then cultured to produce multiple copies of the plasmid.

Penicillin production
Overcoming antibiotic resistance is vital in healthcare. One approach is to develop chemically modified variants of the natural antibiotic. The enzyme penicillin acylase is used to convert natural penicillin G into an intermediate compound, 6-amino penicillanic acid (6-APA), which can be used to produce a range of so-called semisynthetic penicillins.

Lactose-free milk
The presence of lactose in milk produces serious effects in people who are lactose-intolerant. The enzyme lactase is used to remove lactose from milk by converting it to galactose and glucose.

Washing powders
Egg, blood and grease are all targets for enzymes in washing powders. Such powder contains proteases, amylase, lipase and cellulase. The first three help to remove protein, carbohydrate and lipid-based stains; cellulase acts as a conditioner and cleanser for cotton fabrics, removing loose microfibrils (very small fibres) and dirt.

Biological washing powders now occupy a substantial part of the market in Western Europe, having both environmental and energy benefits. They reduce the need for powders with a strong solvent and high phosphate content; their disposal creates fewer environmental problems. Biological washing powders can also be used at relatively low temperatures, resulting in energy saving.

Exam Hint: *Produce a balanced and relevant response to a topic. Develop the proper range of information; do not make an essay on commercial enzymes read like a response to a question on genetic engineering, just because you know a lot about restriction enzymes and DNA.*

High fructose syrups
High fructose syrups are used to replace sucrose as a sweetener in soft drinks and confectionery. The commercial advantage is that fructose is much sweeter than sucrose. Three enzymes are involved – α amylase, amyloglucosidase and glucose isomerase. The raw material is corn starch. Alpha-amylase helps to break the large starch molecules to smaller units called maltodextrins. These are then converted to glucose by amyloglucosidase, which removes glucose units from the ends of the dextrin chains. Glucose isomerase converts glucose to fructose.

Starch —(α amylase)→ Maltodextrins —(amyloglucosidase)→ Glucose

Apple Juice Production
Cold-stored apples develop relatively high levels of soluble pectin because of enzyme changes within the apples during storage. The pectin has a high water-binding capacity and reduces the yield of the pressed juice. The pectin is reduced if the enzyme pectinase is added to the fruit during the crushing stage.

Practice Questions

1. State three advantages of using enzymes commercially compared with other types of catalyst. (3 marks)
2. Describe the commercial use of restriction endonucleases (4 marks)
3. Describe the commercial uses of:
   (a) lipases (3 marks)
   (b) lactases (4 marks)
   (c) pectinases (3 marks)

Answers
(Semicolon indicates marking points)

1. specificity;
   energy saving;
   less pollution;
2. genetic engineering of insulin;
   somatotropin/interferon;
   improving flavour of tomato puree;
   inserting pesticide resistance into plants;
   (credit any valid example)
3. (a) washing powder;
   breaks down lipids;
   to fatty acids and glycerol;
   (b) milk treatment;
   breaks down lactose;
   to galactose and glucose;
   lactose-intolerance;
   (c) apple juice treatment;
   reduces pectin content;
   reduces water-binding capacity of pectin;
   clarifies the juice;

Acknowledgements;
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